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# Pathogenesis of Human Urinary Bladder Cancer

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**The pathogenesis of bladder cancer is being analyzed at several levels of biological organization, i.e., population groups, individual whole animal, tissue, cell, molecule, etc. Each of these levels provides opportunities for mechanistic studies. Yet the integration of these several levels into a cohesive fabric is incomplete. From a clinical point of view, the following seem of importance to human bladder cancer pathogenesis. The initiation, promotion, and progression of bladder cancer involves several factors acting concurrently or sequentially. These factors appear to be naturally occurring or synthetically created chemicals present in the external environment. Human exposures to these agents may begin *in utero*, and varying, dynamic qualitative and quantitative exposure patterns continue through developmental and adult life. Apparent latent periods of development of clinical bladder cancer may be as short as one, or as long as 50 years or more. Individuals may exhibit differential susceptibility to vesical carcinogens, perhaps through phenotypic differences in quantitative biotransformation routes. Differences in bladder epithelial cell susceptibilities probably also occur, as well as varying local tissue and generalized resistance to neoplasia formation. Older individuals do not appear to be more resistant to bladder carcinogenesis. A number of animal model systems have been developed for the study of the *in vivo*, cellular, and molecular pathogenesis of bladder cancer. These models replicate many of the known salient features of human bladder cancer. Through use of appropriate whole animal models in conjunction with investigations of human and animal bladder cells and tissues in culture, controlled mechanistic and quantitative studies of bladder cancer pathogenesis should rapidly develop.**

## Introduction

The pathogenesis of human bladder cancer has been investigated intensively for several decades. Early studies involved descriptions of the development of these neoplasms in workers exposed to occupational arylamines. Latter studies have utilized animal models, and have been extended to tissue, cellular, and molecular investigations. Each of these levels of activity has provided opportunities for mechanistic studies. Yet we still do not have complete analyses of the processes involved in bladder carcinogenesis. In order to devise more rational approaches to the control of this disease, additional studies are urgently required. The purposes of this brief review are to integrate the available information concerning human bladder cancer pathogenesis and to direct attention to areas of further investigation.

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## Historical Considerations

Bladder cancer in most humans appears to be multifactorial in origin and multistage in evolution (*1-8*). Our natural environment may contain a number of factors important in the etiology of bladder cancer (*5, 6, 9*). These factors may have been present on this planet for as long as humans have. For example, bracken fern (BF) (*Pteridium aquilinum*), now known to be a potent plant experimental bladder carcinogen, was used by several tribes of Pacific Northwestern Indians as a dietary staple as long ago as 14,000 B.C. (*10*). It continues to be used as a human food and veterinary feed in many parts of the world (*10, 11*). Schistosomiasis, caused by a parasitic organism, *Schistosoma haematobium*, was connected by early Egyptians about 3,000 B.C. to bladder cancer (*11*). In Egypt and other areas of Africa many individuals from an early age develop repeated infestations by this parasite. Cancer of the urinary bladder is the most common form of cancer in Egypt. It was again suggested in 1911 by Ferguson (*12*) that schistosomiasis was associated with bladder cancer. The role of this parasite, if any, in the etiology of bladder cancer is not clear, although recent data from certain animal studies

seem to support an etiologic relationship (*13*). Tobacco was used for many centuries in tribal ceremonies by North American Indians. The tobacco smoking culture was introduced into Europe in 1519 by Spanish explorers, and its use spread rapidly to Asia and Africa (*8*). As early as 1761, Dr. John Hill reported a relationship between tobacco use and cancer (*8*). However, it remained until the 1950s for the presentation of evidence that cigarette smoking was etiologic for human bladder cancer (*8*). To date, the constituents in tobacco smoke responsible for bladder carcinogenesis are unknown. Thus the deliberate or accidental human exposures to agents in our natural environment are believed to pose bladder carcinogenic hazards.

The urinary bladder was among the first visceral organs for which specific chemicals were identified as causal agents of human cancer (*14*). In 1895 the German clinician Rehn (*15*) suggested that workers employed in the dye industry were at increased risk of bladder cancer. This report was followed over the next 50 years by many reports of other workers in several countries who identified similar clusterings of industrial exposures to aromatic amines and the development of bladder cancer in exposed individuals (*16*). A major and significant epidemiologic study reported in 1954 by Case and his associates (*17*) demonstrated that dye workers were at a 10- to 80-fold increased risk of dying from bladder cancer. The magnitude of risk varied with the particular aromatic amine handled, and with the duration and intensity of exposures. In selected circumstances, as many as 100% of exposed workers developed bladder cancer (*16*). Benzidine and 2-naphthylamine were identified as especially potent human bladder carcinogens (*16*, *17*). 1-Naphthylamine was associated with a low risk, possibly due to contamination with 2-naphthylamine (*16*, *17*). In 1955, evidence was presented by clinical investigators that workers exposed to 4-aminobiphenyl were at increased risk of bladder cancer formation (*18*). Thus, by the mid-1950s, conclusive data were available implicating at least three industrial arylamines as potent human bladder carcinogens (*2*, *6*, *8*, *11*, *16*). On the basis of these data, several industrialized countries took steps to limit or abolish manufacture of these chemicals (*2*, *16*). At that time it was generally believed that no more than 1% of human urothelial neoplasms were etiologically related to exposures to one or more of these chemicals (*16*). Thus, studies by clinicians concerned about causes of bladder cancer in their patients provided important leads for other investigators.

The second major advance in studies of the causation of bladder cancer came in 1938 when Hueper and co-workers (*19*) demonstrated the production of bladder cancer in dogs administered 2-naphthylamine. This major achievement led to the development of methods to investigate in the laboratory under controlled conditions the carcinogenicity of known or suspected chemical bladder carcinogens. It also provided opportunities to investigate the cellular and molecular temporal pathogenesis of bladder cancer in an effort to elucidate these phenomena and construct rational approaches to their inhibition or reversal (*2-4*). Initial work with experimental animal systems progressed slowly, as bladder tumor yields were generally low, latent periods of generation were lengthy and biological evidences of invasive, metastatic, or lethal qualities were often lacking (*3*). Early studies tended to be descriptive rather than mechanistic. During the past twenty years more workers have been attracted to study the causes of bladder cancer, and as a result, significant new data and progress have occurred. Aspects of this topic have been extensively reviewed recently (*1-4*, *6*, *11*, *16*). The term "bladder cancer" as used in this paper is an inclusive term referring to tumors of the renal pelvis, ureter, bladder, and urethra. On the basis of present knowledge, it appears that tumors at these sites probably have similar causes.

## Epidemiological Studies

Incidence rates for bladder cancer in many countries are increasing at variable rates. For example, in the United States these rates per 100,000 population during the period 1950-54 were: males, 14.1 and females, 4.4 (*7*). By 1970-74 comparable incidence rates were: males, 23.7, and females, 6.1 (*7*). Several recent studies have attempted to quantitate the attributable risk percent of factors associated with human bladder cancer causation (*5*, *20*, *21*) (Table 1). These studies (*5*, *20*, *21*) conclusively demonstrate that cigarette smoking is significantly responsible for bladder cancer causation in men, and to a lesser extent, women, in the areas studied. Exposures to agents in hazardous occupations are also responsible for augmented risk of bladder cancer, especially in men. However, factors for a significant fraction of bladder cancers, especially in women, are not known (*5*, *20*, *21*). Attributable risk percent varies with age (*5*). For example, in men in Massachusetts, the attributable risk percent of ages 20-59, 60-74, or 75-89, respectively, are: cigarette smoking, 54, 41, 19; hazardous occupations, 23, 20, 7; and unknown, 23, 39, 74 (*5*). These data suggest that new factors responsible for bladder cancer causation have entered our environment since 1900 and that these may be related to smoking or hazardous occupations. In Massachusetts, the hazardous occupa-

Table 1. Attributable risk per cent of factors associated with human bladder cancer causation.

| | Massachusetts (USA)[a] | | Canada[b] | | England[c] | |
|---|---|---|---|---|---|---|
| | Males | Females | Males | Females | Males | Females |
| Cigarette smoking | 39 | 29 | 56 | 29 | 85 | 27 |
| Hazardous occupations | 18 | 6 | 35 | 1 | | |
| Unknown | 43 | 65 | 9 | 70 | 15 | 73 |

[a]Data of Morrison and Cole (*5*).
[b]Data of Miller (*20*).
[c]Data of Moolgavkar and Stevens (*21*).

Table 2. Accepted human bladder carcinogens.[a]

| Source | Chemical | Experimental bladder carcinogen[b] |
|---|---|---|
| Occupational | 4-Aminobiphenyl | Yes (D, M, Rab.) |
| | Auramine | No |
| | Benzidine | Yes (D) |
| | 2-Naphthylamine | Yes (D, Mon., H, R) |
| Medicinal | Cyclophosphamide | Yes (M, R) |
| | *N,N*-Bis(2-chloroethyl)-2-naphthylamine | No |
| | Phenacetin | Yes (R) |
| Habit – tobacco smoke | Unknown | No |

[a]Cited in the literature (*1, 2, 5, 6, 8, 9, 11, 14, 16*).
[b]D = dog; M = mouse; Rab. = rabbit; Mon. = monkey; H = hamster; R = rat.

tions identified were those associated with dyes, rubber, leather, painting, and other organic chemicals (*5*).

Presented in Table 2 are the sources of exposure, chemical names, and species exhibiting bladder carcinogenesis to chemicals or substances generally accepted as human bladder carcinogens. Provided in Table 3 are available data concerning exposure sources, latency periods, minimum exposure times and estimated relative risks for several accepted human bladder carcinogens. It appears from these data that prolonged exposure to these agents need not necessarily precede the expression of clinical bladder cancer. Does this suggest that brief, intense exposures to potent bladder carcinogens are sufficient to produce clinical disease? Or do these observations suggest that bladders of susceptible individuals harbor cells previously initiated or promoted by other, more ubiquitous bladder trophic agents, and that the final exposure provides a milieu sufficient for clinical malignancy?

From the standpoint of prevention, it is important to ask the following. Does age of initial exposure have any influence on the excess risk of bladder cancer development? In one study (*24*), all of the excess risk of bladder cancer among men with occupational exposures was confined to those whose exposures began prior to age 25. On the other hand, a recent study by Ohkawa and associates (*25*) suggests that Japanese men introduced into benzidine manufacturing industries had a significantly shorter latent period of bladder cancer development after age 40 (14.4 ± 6.0 years) than did men who began working in the same industries prior to age 39 (23.6 ± 9.7 years) ($p < 0.01$). Several other studies (*24*) also indicate that older individuals are more susceptible than younger ones to cancer induction. At this time it does not seem reasonable to suggest that only older workers should be placed in potentially carcinogenic work environments.

Another important issue is duration of exposure. Several studies from occupational sources and from patients exposed to antineoplastic alkylating agent therapy showed that relatively short exposures of 4.5 to 12 months appreciably increased bladder cancer risk (Table 3). Host capabilities to biotransform hazardous procarcinogens to ultimate molecular species, as well as relative susceptibilities of target tissues may be important variables in determining increased susceptibility (*8*).

Other factors in humans that may predispose to bladder malignancies include anatomic abnormalities, e.g., extrophy, patent urachus, diverticulum, etc.; persistent infections, e.g., in young females; schistosomiasis, etc.; urinary sources of natural carcinogens from plant food sources, or abnormal tryptophan metabolism; or pelvic radiation in women (*1, 4-6, 9, 11, 14*). There are significant geographic international variations in bladder cancer incidences of more than 10-fold in males (*26*). For example, reported (*26*) rate variations per 100,000 population were from 28.7 in Bulwayo, Africa to 2.8 in Maori,

Table 3. Exposure sources, latency periods, minimum exposure times, and relative risks for accepted etiologic factors in human bladder carcinogenesis.[a]

| Chemical | Exposure[b] | Latency period, yr | | Minimum exposure time, mo. | Relative risk |
|---|---|---|---|---|---|
| | | Range | Mean | | |
| 4-Aminobiphenyl | Occ. | 15-35 | ?[c] | 4.5 | 200 |
| Auramine | Occ. | 9-28 | ?[c] | 15 | 13 |
| Benzidine | Occ. | 1-45 | 14.4-23.6 | ?[c] | 14 |
| *N,N*-Bis(2-chloroethyl)-2-naphthylamine | Med. | 2.5-10 | 5.5 | ?[c] | ?[c] |
| 2-Naphthylamine | Occ. | 5-45 | 16-18 | 6-12 | 80 |

[a]Cited in the literature (*1, 2, 5, 6, 8, 9, 11, 14, 16, 22-25*).
[b]Occ. = occupational; Med. = medicinal.
[c]Data not available.

New Zealand. Individuals migrating from Japan (a low incidence area) to Hawaii (a high incidence area) developed incidence patterns of bladder cancer intermediate between those native Japanese and U.S whites (*26*). These data suggest that environmental determinants play a major role in bladder cancer causation in humans.

## Suspect Natural Environmental Bladder Carcinogenic Factors

The plant food delicacy BF was associated by Pamukcu as a major factor in the genesis of bovine bladder cancer (*27, 28*). BF and other botanical relatives grow well in moist, shaded areas or hilly pasture lands in temperate and midtropic zones North and South of the equator (*27, 28*). As these ferns are among the first to develop in the spring, they frequently are ingested by grazing domestic animals. In the fall, farmers in less well developed countries harvest BF for use as animal bedding, where it may also be eaten by animals. BF and related plants are produced commercially in Japan, Canada and northeastern parts of the United States for human diets as greens or salads (*27-29*). Milk and dairy products obtained from cows grazing on BF are also consumed by humans. Thus, some humans are directly exposed to BF in their diets and indirectly exposed to BF through products obtained from animals. In several areas of the world an association of BF ingestion has been made with the appearance of urinary bladder tumors in cows and water buffalo (*27, 28*), esophageal and intestinal tumors in cows (*28*) and bladder and intestinal tumors in sheep (*27, 28*). The incidence of bladder tumors may vary from one area to another, but may be as high as 90% (*27, 28*).

Evidence that ingested BF is a primary determinant of bladder cancer in cows stems from several studies (*27, 28*). Deliberate, prolonged low-level 2 g/kg body weight) feeding of BF produced bladder cancers in 20 of 30 cows. A mean period of 550 days from the start of BF feeding to tumor appearance was determined. Invasive bladder carcinomas appeared within 2.5 years and the induced vesical neoplasms were histologically indistinguishable from the naturally occurring bladder tumors. Other species also exhibit a variety of neoplastic lesions when fed BF (*27, 28*). Rats developed vesical carcinoma and ileal adenoma, adenocarcinoma, and sarcoma; mice demonstrated urinary bladder and intestinal carcinoma, pulmonary adenoma and adenocarcinoma, and leukemia; guinea pigs had bladder and intestinal tumors; and Japanese quail displayed intestinal adenocarcinoma following oral administration of BF.

A recent epidemiologic study in Japan suggested that daily intake of BF by humans significantly increased risk of development of esophageal cancer (*29*). When combined with a hot tea gruel (chagayu) drink, the relative esophageal cancer risk was increased in males to 5.2 and in females to 4.7. Addition of cigarette smoking to daily intake of BF and chagayu further increased the relative esophageal cancer risk in men to 9.2 and in women to 6.4. Combined use of BF, chagayu, and cigarettes resulted in a 34-fold higher relative esophageal cancer risk for both sexes. Combined attributable risk of these factors to esophageal cancer development was calculated as 80.2% It was suggested that BF acted as an initiator, and the hot tea gruel as a promoter or localizer of these neoplasms in the human populations studied. Daily intake of meats and fruits tended to lower esophageal cancer risk. Human bladder cancer was not studied (*29*).

The human hazard from BF is not restricted to direct consumption because lactating cows fed BF excreted in their milk a urinary and intestinal tract carcinogen for rats, and a mutagen for *S. typhimurium* TA 100 (*30*). Humans may be exposed to the BF carcinogen present in milk and dairy products where grazing cattle ingest BF, especially under free-range conditions. Infants may be exposed to the BF carcinogen through maternal milk, if the mother consumes BF daily. The BF carcinogen can

pass placental barriers of cows and mice and is excreted in urine of cows and rats fed BF (*27, 28*). Fractions of urine obtained from cows fed BF demonstrated carcinogenic activity when implanted intravesically in mice (27, *28*). Rats fed BF excreted two different biologically active compounds in urine (*31*). One chemical was mutagenic for *S. typhimurium* TA 98 and TA 100 and was identified as quercetin (*31*). The other isolate was mutagenic for TA 100 but not TA 98, and its identification is in progress. We recently have reported the intestinal and bladder carcinogenicity for rats of quercetin (*32*). It appears that BF contains other chemicals as well that are possible bladder carcinogens.

In addition to BF, some other ferns or other plants may also be vesical carcinogens. For example, it was reported (*33*) recently that in the absence of BF, chronic ingestion of mugla fern or rock fern (*Cheilanthes sieberi*) was a major cause of bladder neoplasms in cows residing in Queensland. Hirono and his associates (*34*) reported the development of hepatic and vesical tumors in rats fed Russian comfrey (*Symphytum officinal* L.), used by humans as a green vegetable or tonic. Humans of all ages may consume these plant-derived carcinogens through direct ingestion in food or drinks, in prepared herbal remedies, or through consumption of derived animal products, e.g., milk, cheese, etc. In some instances plant-derived carcinogens structurally resemble synthetic chemical carcinogens; in other cases unique structures have been characterized; and in many circumstances responsible plant carcinogens remain to be isolated and identified (*28*).

The essential amino acid, L-tryptophan, in the course of its metabolism gives rise to a number of urinary primary aromatic amine and quinoline metabolites (*35, 36*). These metabolic pathways are regulated to a great extent by vitamin $B_6$ (pyridoxine) (*35*). Several of these urinary metabolites are excreted in elevated quantities in patients with bladder cancer, and quantitative alterations of metabolism are related to relative deficiencies of vitamin $B_6$ (*35*). Patients with a greater risk of development of heterotopic recurrent bladder cancer were those exhibiting abnormal tryptophan metabolism (*37*). Several urinary metabolites of L-tryptophan displayed bladder oncogenicity (*36*). Recent studies (*38-41*) suggest that L-tryptophan is cocarcinogenic for the bladder and may promote bladder carcinogenesis.

Data from several studies (*5, 20*) have suggested an association between coffee drinking and increased bladder cancer risk. However, other studies have failed to show such an association, and it is not yet known if effects of chance variation account for the discrepant results. Studies of the relationship of coffee drinking and experimental bladder cancer have not been reported.

## Suspect Synthetic Chemical Factors

In addition to the synthetic chemicals discussed above that have been related etiologically to human bladder carcinogenesis, or those chemicals that are produced commercially and are close structural analogs of recognized human bladder carcinogens, more than 50 chemicals have been reported to display bladder carcinogenicity for experimental animals (*1-4, 6, 8, 9, 11, 16, 23*) (Table 4). In some instances, the evidence supporting bladder carcinogenic effects is sparse, stemming from a single study. In other

**Table 4. Examples of chemicals with some experimental evidence for bladder carcinogenesis.[a]**

| Class | Exposures[b] | Representative chemicals |
|---|---|---|
| Amine or azo dyes | Occ.<br>User<br>Environ. | 3,3′-Dimethoxybenzidine, 3,3′-dichlorobenzidine, *o*-tolidine, *p*-cresidine, *o*-anisidine, *o*-toluidine, *o*-aminoazotoluene, diacetylaminoazotoluene, *p*-dimethylaminoazobenzene, citrus red No. 2, oil orange SS, ponceau 3R, Sudan I, Sudan II, *N*-phenyl-2-naphthylamine, 4-amino-2-nitrophenol, 3,2′-dimethyl-4-aminodiphenyl, 2-methoxy-3-aminodibenzofuran, *N*-2-fluorenylacetamide, dimethylazobenzene, 4,4′-methylene-bis(2-chloroaniline), 2-aminofluorene |
| Nitrosamines | Bact. Inf.<br>Foods<br>Bev.<br>Occ. | *N*-Methyl-*N*′-nitrosourea, *N,N*-dibutylnitrosamine, *N*-butyl-*N*-hydroxybutylnitrosamine, *N*-butyl-*N*-(3-carboxypropyl)-nitrosamine, *N*-methyl-*N*-dodecylnitrosamine, *N*-ethyl-*N*-(4-hydroxybutyl)nitrosamine |
| Nitroaryl | Occ.<br>Med. | 4-Nitrobiphenyl, 2-nitronaphthalene, *N*-[4-(5-nitro-2-furyl)-2-thiazolyl]formamide, formic acid 2-[4-(5-nitro-2-furyl)-2-thiazolyl]hydrazide, *N*-[4-(5-nitro-2-furyl)-2-thiazolyl]acetamide, 2-amino-4-(5-nitro-2-furyl)thiazole |
| Miscellaneous | Occ.<br>Med. | 4-Ethylsulfonylnaphthalene-1-sulfonamide, diethylene glycol, 3-phenyl-5β-dimethylaminoethyl-1,2,4-oxadiazole, 4′-hydroxy-2,3′-dimethylazobenzene, 2(*N*-butyloxycarbonylmethylene)-thiazolid-4-one, 2-*p*-methoxybenzenesulfonamido-5-isobutyl-1,3,4-thiadiazole, phenacetin, 4-ethoxyphenylurea, saccharin, cyclamate, quercetin |

[a]Cited in the literature (*1-4, 6, 8, 9, 11, 16, 23*).

[b]Occ. = occupational; Environ. = environmental; Bact. Inf. = bacterial infection; Bev. = beverage; Med. = medicinal.

circumstances the evidence is strong, supported by multiple studies or consistent observations in several species. Some of these chemicals, e.g., *N*-[4-(5-nitro-2-furyl)-2-thiazolyl]formamide or *N*-butyl-*N*-[hydroxybutylnitrosamine, have been used to develop animal models of experimental bladder carcinogenesis (*3, 4, 39*). Human exposures to these chemicals is quite variable. Some of these chemicals are used only in scientific laboratories and may provide potential exposures only to the persons working with them (*3*). With others, occupational, distributive, or consumer exposures may be significant. In other cases, as with the artificial sweeteners, cyclamate and saccharin, commercial application, distribution, or consumption involve the majority of individuals in a given society (*42*). Such widespread usage creates much difficuly in the design of appropriate epidemiological studies in human populations. Both cyclamate and saccharin have evidenced bladder carcinogenicity for rats and mice exposed under a variety of circumstances (*42*). Additionally, they promote the action of known vesical carcinogens in the bladders of both sexes of rats (*39, 40, 42*). Available epidemiological data suggest that human exposure to saccharin provides a small risk of bladder carcinogenesis in the general population, or a larger increased risk in some persons with high exposures or confounding exposures to other bladder carcinogens such as cigarettes or perhaps coffee (*42*). As products containing artificial sweeteners are widely consumed by people, and since only 20 years has occurred since their generalized commercial introduction, it still seems prudent to recommend that caution be employed in the indiscriminate or long-term use of these products, especially by children and young adults.

## Future Directions

Despite the progress that has been made in understanding the pathogenesis of human and experimental bladder carcinogenesis, many opportunities for future studies remain. The control of this and other elusive neoplastic diseases can profitably be approached by a dissection of pathogenic mechanisms into their identifiable component processes. Through such analyses a comprehensive process-orientated understanding will enable us to devise mechanisms of control. Advocacy of cessation of smoking might be anticipated to substantially reduce bladder, pulmonary, and certain other neoplasms as major disease problems. Curtailment of usage of certain occupational bladder carcinogens must continue to be a part of public policy.

Refinements of methods for the identification of bladder carcinogens and the investigation of their complex interactions are required. A major difficulty in human risk assessment is related to extrapolation of data from animals to humans; improved methods of study are needed here. Dose-response relationships are incompletely understood in risk analysis. Are there "threshold" doses for bladder carcinogens? Does the addition of other carcinogens or noncarcinogens alter this? A substantial effort to educate practicing physicians and nonscientists to the concepts of carcinogenesis and to the opportunities to apply these concepts to the prevention of cancer is required. Through these multiple approaches implementation of preventative measures can be sought for the betterment of all.

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